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=> d his

     (FILE 'HOME' ENTERED AT 10:13:14 ON 08 JUN 2005)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, WPIDS, SCISEARCH, AGRICOLA'
     ENTERED AT 10:14:05 ON 08 JUN 2005
L1       250658 S (FUS? OR CHIMER? OR CHIMAER?)(5A)PROTEIN?
L2        18958 S HETEROLOG?(5A)PROTEIN?
L3       265896 S L1 OR L2
L4          660 S L3 (5A)STAPH?(5A)PROTEIN(A)A
L5          361 S L3 (5A)FC(5A)PROTEIN(A)A
L6         1002 S L4 OR L5
L7          190 S L6 (5A)EXPRESS?
L8           17 S L6 (5A)(DNA OR RNA OR RIBONUCLEIC OR DEOXYRIBONUCLEIC OR NUC
L9          313 S L6 AND (DNA OR RNA OR RIBONUCLEIC OR DEOXYRIBONUCLEIC OR NUC
L10          42 S L6 (5A)ENCOD?
L11         430 S L7-L10
L12        4624 S PROTEIN(A)A (5A)AFFINITY
L13           8 S L12 AND L11
L14          46 S L11 AND AFFINITY(5A)CHROMATOGR?
L15          53 S L11 AND AFFINITY(5A)PURIF?
L16          36 S L11 AND AFFINITY(5A)BIND?
L17          97 S L14-L16
L18         581 S L3(5A)CALMODULIN
L19          75 S L18 (5A)EXPRESS?
L20         148 S L18 AND (DNA OR RNA OR RIBONUCLEIC OR NUCLEIC OR DEOXYRIBONU
L21           2 S L18 (5A)ENCOD?
L22         201 S L19-L21
L23          28 S L22 AND AFFINITY(5A)CHROMATOGR?
L24          33 S L22 AND AFFINITY(5A)PURIF?
L25          38 S L22 AND AFFINITY(5A)BIND?
L26          64 S L23-L25
L27         160 S L17 OR L26
L28          40 S L27 AND CLEAVAGE
L29          42 S L27 AND CLEAV?
L30         626 S SERAPHIN B?/AU
L31          50 S RIGAUT G?/AU
L32         644 S L30 OR L31
L33           7 S L32 AND STAPH? (5A)PROTEIN(A)A
L34           1 S L27 AND TOBACCO(5A)ETCH
L35          15 S L27 AND PROTEOLY?
L36           6 S L27 AND PROTEASE?
L37           5 S L27 AND PROTEINASE?
L38          64 S L29 OR L33 OR L34 OR L35 OR L36 OR L37
L39          24 DUP REM L38 (40 DUPLICATES REMOVED)

=> d ibib abs l39 1-24

L39  ANSWER 1 OF 24  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:         2003:244781  HCAPLUS
DOCUMENT NUMBER:          138:283688
TITLE:                    Nucleic acids, vectors, fusion proteins and
                          method for detection and identification of protein
                          ligands
INVENTOR(S):              Bonneu, Marc; Crouzet, Marc
PATENT ASSIGNEE(S):       Universite Victor Segalen Bordeaux 2, Fr.
SOURCE:                   Fr. Demande, 74 pp.
                          CODEN: FRXXBL
DOCUMENT TYPE:            Patent
LANGUAGE:                 French
```

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 2830020	A1	20030328	FR 2001-12337	20010925
FR 2830020	B1	20031219		
PRIORITY APPLN. INFO.:			FR 2001-12337	20010925

AB A **nucleic** acid encoding a marker protein (such as GFP) fused to an **affinity purification** peptide, a chimeric gene consisting of the **nucleic** acid fused to a bait protein, and vectors containing such chimeric genes are disclosed. Cells transformed with such vectors may be used for localization of the ligand(s) of the bait protein. Alternatively, the cell may be lysed and the complex composed of the bait protein-GFP-affinity peptide and ligand may be isolated by affinity chromatog. and may be characterized by gel exclusion chromatog. Thus, a **fusion protein** comprising Ade4p, GFP, **calmodulin**-binding peptide, and hexahistidine was **expressed** in yeast. A 320-kDa complex was identified by gel filtration and **purified** on calmodulin and nickel ion **affinity** columns. The 320-kDa complex was found to be a tetramer of the Adep-GFP fusion protein.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L39 ANSWER 2 OF 24 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2002264572 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12003931
TITLE: Identification and physical characterization of the HbpR binding sites of the hbpC and hbpD promoters.
AUTHOR: Tropel David; van der Meer Jan Roelof
CORPORATE SOURCE: Process of Environmental Microbiology and Molecular Ecotoxicology, Swiss Federal Institute for Environmental Science and Technology (EAWAG), CH-8600 Dubendorf, Switzerland.
SOURCE: Journal of bacteriology, (2002 Jun) 184 (11) 2914-24. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200206
ENTRY DATE: Entered STN: 20020511
Last Updated on STN: 20020611
Entered Medline: 20020607

AB Pseudomonas azelaica HBP1 can use 2-hydroxybiphenyl (2-HBP) and 2,2'-dihydroxybiphenyl as sole carbon and energy sources by means of the hbp regulon. This regulon is composed of three genes, hbpCA and hbpD, coding for enzymes of a meta-**cleavage** pathway and the hbpR gene, which codes for a XylR/DmpR-type transcription regulator. It was previously shown that HbpR activates transcription from two sigma(54)-dependent promoters, P(hbpC) and P(hbpD), in the presence of 2-HBP. In this study, by using gel mobility shift assays with a purified **fusion protein** containing **calmodulin** binding **protein** (CBP) and HbpR, we detected two binding regions for HbpR in P(hbpC) and one binding region in P(hbpD). DNase I footprints of the proximal binding region of P(hbpC) and of the binding region in P(hbpD) showed that CBP-HbpR protected a region composed of two inverted repeat sequences which were homologous to the binding sites identified for XylR. Unlike the situation in the XylR/P(u) system, we observed simultaneous

binding of CBP-HbpR on the two upstream activating sequences (UASs). Fragments with only one UAS did not show an interaction with HbpR, indicating that both pairs of UASs are needed for HbpR binding. The addition of both ATP and 2-HBP increased the **DNA binding affinity** of HbpR. These results showed for the first time that, for regulators of the XylR/DmpR type, the effector positively affects the recruitment of the regulatory protein on the enhancer **DNA**.

L39 ANSWER 3 OF 24 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002417516 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12172685
TITLE: Purification method for recombinant proteins based on a **fusion** between the target **protein** and the C-terminus of **calmodulin**.
AUTHOR: Schauer-Vukasinovic Vesna; Deo Sapna K; Daunert Sylvia
CORPORATE SOURCE: Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506-0055, USA.
CONTRACT NUMBER: GM 47915 (NIGMS)
SOURCE: Analytical and bioanalytical chemistry, (2002 Jul) 373 (6) 501-7. Electronic Publication: 2002-06-29.
Journal code: 101134327. ISSN: 1618-2642.
(Investigators: Daunert S, U KY, Lexington)
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Space Life Sciences
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 20020813
Last Updated on STN: 20030105
Entered Medline: 20020924

AB Calmodulin (CaM) was used as an **affinity** tail to facilitate the **purification** of the green fluorescent protein (GFP), which was used as a model target protein. The protein GFP was fused to the C-terminus of CaM, and a factor Xa **cleavage** site was introduced between the two proteins. A CaM-GFP fusion protein was expressed in E. coli and purified on a phenothiazine-derivatized silica column. CaM binds to the phenothiazine on the column in a Ca(2+)-dependent fashion and it was, therefore, used as an **affinity** tail for the **purification** of GFP. The fusion protein bound to the affinity column was then subjected to a **proteolytic** digestion with factor Xa. Pure GFP was eluted with a Ca(2+)-containing buffer, while CaM was eluted later with a buffer containing the Ca(2+)-chelating agent EGTA. The purity of the isolated GFP was verified by SDS-PAGE, and the fluorescence properties of the purified GFP were characterized.

L39 ANSWER 4 OF 24 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001496886 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11545275
TITLE: Lactobacillus casei acquires the binding activity to fibronectin by the expression of the fibronectin binding domain of Streptococcus pyogenes on the cell surface.
AUTHOR: Kushiro A; Takahashi T; Asahara T; Tsuji H; Nomoto K; Morotomi M
CORPORATE SOURCE: Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo, Japan.. akira-kushiro@yakult.co.jp
SOURCE: Journal of molecular microbiology and biotechnology, (2001 Oct) 3 (4) 563-71.
Journal code: 100892561. ISSN: 1464-1801.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20010910
Last Updated on STN: 20020305
Entered Medline: 20020304

AB Fibronectin binding domain was expressed on the cell surface of Lactobacillus casei strain Shirota which hardly adheres to fibronectin. **DNA** for the fibronectin binding domain of the sfbl gene, which encodes a fibronectin binding protein of Streptococcus pyogenes ATCC 21059, was amplified with polymerase chain reaction, cloned into a surface display vector pSAK332, and introduced into L. casei. The fibronectin binding domain was **expressed** as **a fusion protein** consisting of **staphylokinase** of **Staphylococcus** aureus and the anchor sequence of cell wall-associated 763 **proteinase** of Lactococcus lactis NCDO 763. The fibronectin binding ability of the resulting L. casei was confirmed with Western blot analysis, immunoelectron microscopic analysis, and adherence to fibroblast cells. These results indicate that L. casei has acquired a new phenotype to bind fibronectin upon the expression of the fibronectin binding domain on the cell surface. This L. casei also shows **binding affinity** to fibrinogen, indicating that fibronectin **binding** domain is involved in the binding to fibrinogen as well.

L39 ANSWER 5 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2001248911 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11302706
TITLE: Stoichiometry of the Sm proteins in yeast spliceosomal snRNPs supports the heptamer ring model of the core domain.
AUTHOR: Walke S; Bragado-Nilsson E; **Seraphin B**; Nagai K
CORPORATE SOURCE: Laboratory of Molecular Biology, MRC, Hills Road, Cambridge, CB2 2QH, UK.
SOURCE: Journal of molecular biology, (2001 Apr 20) 308 (1) 49-58. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20010517
Entered Medline: 20010510

AB Seven Sm proteins (B/B', D1, D2, D3, E, F and G proteins) containing a common sequence motif form a globular core domain within the U1, U2, U5 and U4/U6 spliceosomal snRNPs. Based on the crystal structure of two Sm protein dimers we have previously proposed a model of the snRNP core domain consisting of a ring of seven Sm proteins. This model postulates that there is only a single copy of each Sm protein in the core domain. In order to test this model we have determined the stoichiometry of the Sm proteins in yeast spliceosomal snRNPs. We have constructed seven different yeast strains each of which produces one of the Sm proteins tagged with a calmodulin-binding peptide (CBP). Further, each of these strains was transformed with one of seven different plasmids coding for one of the seven Sm proteins tagged with protein A. When one Sm protein is expressed as a CBP-tagged protein from the chromosome and a second protein was produced with a protein A-tag from the plasmid, the protein A-tag was detected strongly in the fraction bound to calmodulin beads, demonstrating that two different tagged Sm proteins can be assembled into functional snRNPs. In contrast when the CBP and protein A-tagged forms of

the same Sm protein were co-expressed, no protein A-tag was detectable in the fraction bound to calmodulin. These results indicate that there is only a single copy of each Sm protein in the spliceosomal snRNP core domain and therefore strongly support the heptamer ring model of the spliceosomal snRNP core domain.
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L39 ANSWER 6 OF 24 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2000-206012 [18] WPIDS
DOC. NO. CPI: C2000-063725
TITLE: Detection and purification of substances using a **Staphylococcus protein A** affinity tag, useful for studying the structure, activities or interactions of proteins or for identifying potential drugs.
DERWENT CLASS: D16
INVENTOR(S): **RIGAUT, G; SERAPHIN, B**
PATENT ASSIGNEE(S): (EUMO-N) EURO LAB MOLEKULARBIOLOGIE; (EMBL-N) EMBL EURO LAB MOLEKULARBIOLOGIE
COUNTRY COUNT: 89
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000009716	A1	20000224	(200018)*	EN	31
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9957362	A	20000306	(200030)		
EP 1105508	A1	20010613	(200134)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
US 2002061513	A1	20020523	(200239)		
EP 1231276	A1	20020814	(200261)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
JP 2002522085	W	20020723	(200263)		29
EP 1105508	B1	20020904	(200266)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
DE 69902796	E	20021010	(200274)		
ES 2183601	T3	20030316	(200325)		
AU 762961	B	20030710	(200355)		
AU 2003252910	A1	20031106	(200432)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000009716	A1	WO 1999-EP6022	19990817
AU 9957362	A	AU 1999-57362	19990817
EP 1105508	A1	EP 1999-944420	19990817
		WO 1999-EP6022	19990817
US 2002061513	A1 Cont of	WO 1999-EP6022	19990817
		US 2001-785793	20010216
EP 1231276	A1 Div ex	EP 1999-944420	19990817
		EP 2002-4765	19990817
JP 2002522085	W	WO 1999-EP6022	19990817
		JP 2000-565150	19990817

EP 1105508	B1		EP 1999-944420	19990817
			WO 1999-EP6022	19990817
		Related to	EP 2002-4765	19990817
DE 69902796	E		DE 1999-602796	19990817
			EP 1999-944420	19990817
			WO 1999-EP6022	19990817
ES 2183601	T3		EP 1999-944420	19990817
AU 762961	B		AU 1999-57362	19990817
AU 2003252910	A1		AU 2003-252910	20031010

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9957362	A	Based on	WO 2000009716
EP 1105508	A1	Based on	WO 2000009716
EP 1231276	A1	Div ex	EP 1105508
JP 2002522085	W	Based on	WO 2000009716
EP 1105508	B1	Related to	EP 1231276
		Based on	WO 2000009716
DE 69902796	E	Based on	EP 1105508
		Based on	WO 2000009716
ES 2183601	T3	Based on	EP 1105508
AU 762961	B	Previous Publ.	AU 9957362
		Based on	WO 2000009716
AU 2003252910	A1	Div ex	AU 762961

PRIORITY APPLN. INFO: EP 1998-115448 19980817

AN 2000-206012 [18] WPIDS

AB WO 200009716 A UPAB: 20000412

NOVELTY - A method for detecting or purifying substances uses polypeptides or subunits fused to at least 2 different affinity tags, at least one of which is an IgG binding domain of **Staphylococcus protein A** (SPA).

DETAILED DESCRIPTION - A method of detecting and/or purifying substances selected from proteins, biomolecules, complexes of proteins or biomolecules, subunits, cell components, cell organelles and cells, comprises:

(a) providing an expression environment containing one or more heterologous nucleic acids encoding polypeptides and/or subunits of a biomolecule complex, the polypeptides or subunits being fused to at least 2 different affinity tags, one of which consists of one or more IgG binding domains of SPA;

(b) maintaining the expression environment to express the polypeptides or subunits in a native form as fusion proteins with the affinity tags; and

(c) detecting and/or purifying the polypeptide or subunits by a combination of at least 2 affinity purification steps, each comprising binding the polypeptides or subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the polypeptides or subunits from the support material after unbound substances have been removed.

INDEPENDENT CLAIMS are also included for the following:

(1) a method for detecting and/or purifying biomolecule and/or protein complexes comprising:

(a) providing an expression environment containing one or more heterologous nucleic acids encoding at least 2 subunits of a biomolecule complex, each being fused to at least one of different affinity tags, one of which consists of one or more IgG binding domains of SPA;

(b) maintaining the expression environment to facilitate expression

of the one or more subunits in a native form as fusion proteins with the affinity tags, and to allow formation of a complex between the one or more subunits and possibly other components capable of complexing with the one or more subunits; and

(c) detecting and/or purifying the complex by a combination of at least 2 different affinity purification steps each comprising binding the one or more subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after unbound substances have been removed;

(2) fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least 2 different affinity tags, where one of the affinity tags consists of at least one IgG binding domain of SPA;

(3) nucleic acid coding for a fusion protein of (2);

(4) a vector comprising a nucleic acid as in (3) under the control of sequences facilitating the expression of a fusion protein as in (2);

(5) a vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least 2 different affinity tags, one consisting of one or more IgG binding domains of **Staphylococcus** aureus **protein A** (SAPA), and at least one PN linker for the insertion of further nucleic acids;

(6) a vector comprising heterologous nucleic acid sequences in form of 2 or more cassettes each comprising at least one of different affinity tags, one consisting of one or more IgG binding domains of SAPA, and at least one PN linker for the insertion of further nucleic acids;

(7) a cell containing a nucleic acid of (3) or a vector of (4); and

(8) a reagent kit comprising a nucleic acid of (3) or a vector of (4), (5) or (6) for the expression of a fusion protein of (2) and support materials each capable of specifically binding one of the affinity tags.

USE - The methods can be used for the detection and/or purification of substances capable of complexing with the fusion protein (claimed). They can also be used for the detection and/or purification of cells and/or cell organelles expressing the fusion protein on their surface (claimed). They can be used for studying the structure, activities or interactions with proteins or nucleic acids. The methods not only facilitate efficient purification of proteins of interest but also allows fishing for and detecting components present in complexes with which the polypeptides or subunits are associated or complexed either directly or indirectly, e.g. molecules such as linker mediators. This would allow selective fishing, for certain substances which may be potential drugs, even from complex mixtures.

Dwg.0/3

L39 ANSWER 7 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER:	1999:683635 HCAPLUS
DOCUMENT NUMBER:	132:31381
TITLE:	In the Laboratory: A generic protein purification method for protein complex characterization and proteome exploration
AUTHOR(S):	Rigaut, Guillaume; Shevchenko, Anna; Rutz, Berthold; Wilm, Matthias; Mann, Matthias; Seraphin, Bertrand
CORPORATE SOURCE:	Biol. Lab., Heidelberg, D-69117, Germany
SOURCE:	Nature Biotechnology (1999), 17(10), 1030-1032 CODEN: NABIF9; ISSN: 1087-0156
PUBLISHER:	Nature America
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB We have developed a generic procedure to purify proteins expressed at their natural level under native conditions using a novel tandem **affinity purification** (TAP) tag. The TAP tag allows the rapid purification of complexes from a relatively small number of cells without

prior knowledge of the complex composition, activity, or function. Combined with mass spectrometry, the TAP strategy allows for the identification of proteins interacting with a given target protein. The TAP method has been tested in yeast but should be applicable to other cells or organisms.
REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L39 ANSWER 8 OF 24 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999288207 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10336854
TITLE: An Escherichia coli expression vector that allows recovery of proteins with native N-termini from purified calmodulin-binding peptide fusions.
AUTHOR: Wyborski D L; Bauer J C; Zheng C F; Felts K; Vaillancourt P
CORPORATE SOURCE: Stratagene Cloning Systems, 11011 North Torrey Pines Road, La Jolla, California 92037, USA.
SOURCE: Protein expression and purification, (1999 Jun) 16 (1) 1-10.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U86347
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990727
Last Updated on STN: 19990727
Entered Medline: 19990712

AB We describe a T7-based Escherichia coli **expression** vector in which **protein** coding sequence is seamlessly **fused** to the N-terminal **calmodulin**-binding peptide (CBP) purification tag. We combined the use of the site-specific **protease** enterokinase (EK) and the type IIs restriction enzyme Eam1104 I, which **cleave** outside their respective (amino acid and nucleotide) target sequences, such that any amino acid sequence may be fused directly C-terminal to the EK-**cleavage** site without codon constraints conferred by the cloning method. PCR products are cloned using ligation-dependent or ligation-independent methods with high cloning efficiencies (>10(6) cfu/microg vector), allowing production of insert quantities sufficient for several cloning experiments with a limited number of PCR cycles, resulting in a significant time-savings and reduced likelihood of accumulating PCR-derived mutations. CBP fusion proteins are expressed to high levels when the CBP peptide is positioned at the N-terminus. CBP **binds** to **calmodulin** with nanomolar **affinity**, and **fusion proteins** are **purified** to near homogeneity from crude extracts with one pass through calmodulin **affinity** resin using gentle **binding** and elution conditions. We show high efficiency seamless cloning of three inserts into the pCAL-n-EK vector, including one encoding the protein c-Jun N-terminal kinase (JNK). CBP-EK-JNK fusion protein was synthesized to 10-20 mg/liter culture and purified to near homogeneity in one step with calmodulin affinity resin. The fusion tag was efficiently removed with EK to yield active JNK with native N-terminal amino acid sequence. Copyright 1999 Academic Press.

L39 ANSWER 9 OF 24 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1998451821 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9778799
TITLE: New constructs and strategies for efficient PCR-based gene manipulations in yeast.

AUTHOR: Puig O; Rutz B; Luukkonen B G; Kandels-Lewis S; Bragado-Nilsson E; **Seraphin B**
CORPORATE SOURCE: EMBL, Heidelberg, Germany.
SOURCE: Yeast (Chichester, England), (1998 Sep 15) 14 (12) 1139-46. Journal code: 8607637. ISSN: 0749-503X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981223

AB Gene disruption and tagging can be achieved by homologous recombination in the yeast genome. Several PCR-based methods have been described towards this end. However these strategies are often limited in their applications and/or their efficiencies and may be technically demanding. Here we describe two plasmids for C-terminal tagging of proteins with the IgG binding domain of the **Staphylococcus** aureus **protein A**. We also present simple and reliable strategies based on PCR to promote efficient integration of exogenous DNA into the yeast genome. These simple methods are not limited to specific strains or markers and can be used for any application requiring homologous recombination such as gene disruption and epitope tagging. These strategies can be used for consecutive introduction of various constructs into a single yeast strain.

L39 ANSWER 10 OF 24 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 1998075925 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9415443
TITLE: Use of protein A gene fusions for the analysis of structure-function relationship of the transactivator protein C of bacteriophage Mu.
AUTHOR: De A; Paul B D; Ramesh V; Nagaraja V
CORPORATE SOURCE: Centre for Genetic Engineering, Indian Institute of Science, Bangalore.
SOURCE: Protein engineering, (1997 Aug) 10 (8) 935-41. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980211

AB A sensitive dimerization assay for **DNA** binding proteins has been developed using gene fusion technology. For this purpose, we have engineered a gene **fusion** using **protein A** gene of **Staphylococcus** aureus and C gene, the late gene transactivator of bacteriophage Mu. The C gene was fused to the 3' end of the gene for protein A to generate an A-C fusion. The overexpressed fusion protein was purified in a single step using immunoglobulin **affinity chromatography**. Purified fusion protein exhibits **DNA** binding activity as demonstrated by electrophoretic mobility shift assays. When the fusion protein A-C was mixed with C and analyzed for **DNA** binding, in addition to C and A-C specific complexes, a single intermediate complex comprising of a heterodimer of C and A-C fusion proteins was observed. Further, the protein A moiety in the fusion protein A-C does not contribute to **DNA** binding as demonstrated by **proteolytic cleavage** and circular

dichroism (CD) analysis. The assay has also been applied to analyze the **DNA** binding domain of C protein by generating fusions between protein A and N- and C-terminal deletion mutants of C. The results indicate a role for the region towards the carboxy terminal of the protein in **DNA** binding. The general applicability of this method is discussed.

L39 ANSWER 11 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1997:728557 HCAPLUS
DOCUMENT NUMBER: 128:12463
TITLE: A TNF receptor antagonistic scFv, which is not secreted in mammalian cells, is expressed as a soluble mono- and bivalent scFv derivative in insect cells
AUTHOR(S): Brocks, Bodo; Rode, Hans-Jurgen; Klein, Michaela; Gerlach, Elke; Dubel, Stefan; Little, Melvyn; Pfizenmaier, Klaus; Moosmayer, Dieter
CORPORATE SOURCE: Institute of Cell Biology and Immunology, University of Stuttgart, Stuttgart, D-70569, Germany
SOURCE: Immunotechnology (1997), 3(3), 173-184
CODEN: IOTEER; ISSN: 1380-2933
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Single chain antibodies (scFv) are usually produced in E. coli, but generation of certain scFv derivs., such as complex fusion proteins or glycosylated forms of scFv is restricted to eukaryotic expression systems. The authors investigated the production of soluble mono- and bivalent single chain antibodies (scFv) in eukaryotic cells and describe a cassette vector system for mammalian and baculovirus expression which is compatible with an established vector system for bacterial expression and phage display selection of scFvs. The applied model scFv was derived from a murine antibody (H398) against human tumor necrosis factor receptor 1 (TNFR60), known to be a potent antagonist of TNF action in its monomeric form and a potential therapeutic agent for treatment of TNF-mediated diseases. Surprisingly, the monomeric scFv form of H398 (scFv H398) is expressed but not secreted in different mammalian cells. In contrast, in insect cells using recombinant baculovirus, a monovalent scFv H398 and a bivalent scFv **fusion protein** with **a** human IgG1 **Fc** region were **expressed** and secreted with correctly processed signal sequence. Concerning the influence of valency of the model Ab and its derivs. on antigen **binding affinity** and neutralization of TNF activity, the authors found that the mono- and bivalent form of scFv H398 possesses the same characteristics as **proteolytically** produced Fab H398 and original mAb H398, resp. Furthermore, fusion of the Ig Fc protein to scFv H398 increase the in vitro half-life at 37°. Thus, the described cassette vectors readily allow the eukaryotic expression of mono- and bivalent scFv derivs. to analyze the influence of valency of scFv mols. on antigen binding and biol. activity.
REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L39 ANSWER 12 OF 24 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 97199368 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9047344
TITLE: A new expression vector for high level protein production, one step purification and direct isotopic labeling of **calmodulin**-binding peptide **fusion proteins**.
AUTHOR: Zheng C F; Simcox T; Xu L; Vaillancourt P

CORPORATE SOURCE: Stratagene Cloning Systems, La Jolla, CA 92037, USA.
SOURCE: Gene, (1997 Feb 20) 186 (1) 55-60.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U36454
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970414
Last Updated on STN: 19980206
Entered Medline: 19970331

AB Calmodulin-binding peptide (CBP), a peptide of 26 amino acids derived from muscle myosin light chain kinase (MLCK), **binds** to calmodulin with nanomolar **affinity**. Proteins fused in frame with CBP can be purified from crude E. coli lysates in a single step using calmodulin **affinity chromatography** (Stofko-Hahn et al., 1992). Because the binding between CBP and **calmodulin** is calcium-dependent, the **fusion protein** can be eluted from the resin with virtually any buffer containing EGTA (2 mM) and used directly for many applications. To take full advantage of this **affinity purification** system, we constructed the versatile CBP fusion protein expression vector pCAL-n. The CBP coding sequence was positioned for fusion at the N-terminus, an advantage that ensures consistent high level synthesis of fusion proteins due to the efficient translation of the CBP in E. coli. The production of fusion proteins from pCAL-n is controlled by the tightly regulated T7(lac)O promoter. A versatile multiple cloning site (MCS) was included to facilitate the cloning of genes of interest. The protein coding sequence for the enzyme c-Jun N-terminal kinase (JNK) was inserted into the MCS of pCAL-n, and the resulting fusion protein CBP-JNK synthesized in E. coli cells at 15-20 mg/l culture. CBP-JNK was purified to near homogeneity in one step with calmodulin affinity resin. Purified CBP-JNK is fully active, and the CBP peptide tag can be removed by **cleavage** with thrombin. We also show that CBP can be efficiently phosphorylated by cAMP-dependent protein kinase. Hence, the purified fusion proteins can be labeled directly with [gamma-32P]ATP and used to probe protein-protein or protein-**nucleic** acid interactions.

L39 ANSWER 13 OF 24 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 97090687 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8936595
TITLE: A Trypanosoma cruzi polyantigen obtained by gene fusion: its expression in Staphylococcus aureus and rapid purification.
AUTHOR: Moreno J I
CORPORATE SOURCE: Division of Molecular Biology and Biochemistry, School of Biological Sciences, University of Missouri-Kansas City 66160-7410, USA.
SOURCE: Protein expression and purification, (1996 Nov) 8 (3) 332-40.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970321
Last Updated on STN: 19970321
Entered Medline: 19970313

AB In order to simplify the large-scale production of three different Trypanosoma cruzi antigens with significant diagnosis value, their coding **DNA** fragments were fused to produce a single molecule. This tripartite **protein** was **expressed** using **a** shuttle **Staphylococcal protein A** (SPA) gene **fusion** vector. The resulting fusion protein location was intracellular when synthesized in Escherichia coli but was also **proteolytically** degraded during its purification. When the same construct was expressed using the Staphylococcus aureus secretion system, a nondegraded expression product was obtained from the culture medium. A "size effect" seemed to take place in the final yield. The SPA tripartite antigen fusion protein was purified in one step using IgG-Sepharose **affinity chromatography**. The SPA affinity tail was removed by specific **proteolysis** with enterokinase and further chromatography on IgG Sepharose. Specific antibodies against individual antigens reacted equally well with the purified tripartite antigen. These results suggest that the simultaneous production of several antigens in a single molecule using the S. aureus secretion system could be a good alternative, when a mixture of cloned antigens is necessary for a strict diagnosis or for immunization experiments.

L39 ANSWER 14 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1995:643286 HCAPLUS
DOCUMENT NUMBER: 123:54154
TITLE: Chimeric receptors containing IgG-binding domains from either protein A or protein G
INVENTOR(S): Lee, Young Moo; Talib, Sohel; Okarma, Thomas B.
PATENT ASSIGNEE(S): Applied Immune Sciences, Inc., USA
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9506125	A1	19950302	WO 1994-US9141		19940823
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN					
RW: KE, MW, SD, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG					
AU 9475635	A1	19950321	AU 1994-75635		19940823
PRIORITY APPLN. INFO.:			US 1993-110653	A	19930823
			WO 1994-US9141	W	19940823

AB Synthetic genes encoding one of the IgG Fc-binding domains of proteins A and G are prepared for use in the construction of chimeric genes. These genes may encode proteins useful in the assay or purification of IgG and in the treatment of autoimmune disease and immunotherapy. A gene encoding the IgG binding domains of protein G, and a gene coding for a receptor containing one IgG binding domain of Protein A and of Protein G, were chemical synthesized. Genes were constructed from three cassettes each encoding a different domain. Synthetic protein G (SG) and chimeric protein BG (SBG) were inserted into a plasmid vector containing a tac promoter and an ampicillin-resistance gene; allowing both amplification and expression in E. coli. In addition to the IgG binding sites, engineered Fc receptors contain a proline-rich, hydrophilic carboxyl terminus providing for immobilization to solid support, and a factor Xa **cleavable** site providing for their use in isolation and **affinity purifn**

. of other genes cloned to the 5' end of these genes. The genes were expressed in E. coli; the biol. activity of the proteins was demonstrated by immunoblotting with human IgG as well as by a competitive enzyme-linked immunoadsorbent assay with different subclasses of human IgG.

L39 ANSWER 15 OF 24 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 94352971 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8073041
TITLE: A single **Fc** binding domain--alkaline phosphatase gene **fusion expresses a protein** with both IgG binding ability and alkaline phosphatase enzymatic activity.
AUTHOR: Wang C L; Huang M; Wesson C A; Birdsell D C; Trumble W R
CORPORATE SOURCE: Department of Bacteriology/Biochemistry, University of Idaho, Moscow 83844.
SOURCE: Protein engineering, (1994 May) 7 (5) 715-22.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941006
Last Updated on STN: 19941006
Entered Medline: 19940928

AB A recombinant gene fusion was created and cloned using a previously constructed gene encoding a monodomain IgG Fc binding protein and the gene coding for bacterial alkaline phosphatase. The construct was able to express and secrete a fusion protein that exhibited both IgG binding and alkaline phosphatase enzymatic activities. Greater than 60% of the protein demonstrating both biological activities was detected from periplasmic space preparations. Nanogram concentrations of the Fc binding--alkaline phosphatase fusion protein allowed primary IgG antibody detection without the use of conjugated secondary antibodies. Removal of the domain coding for alkaline phosphatase resulted in decreased resistance of the protein to **proteolytic** degradation and the loss of IgG Fc binding ability. Using **affinity-purified** fusion protein, the specificity of binding to IgG, IgM and IgA was examined; binding was strong to IgG and barely detectable against IgM or IgA. **Affinity** for **binding** of the fusion protein to IgG (Kd = 6.7 x 10(-8) M) was determined to be equal to or greater than previously reported for protein A.

L39 ANSWER 16 OF 24 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 94:297724 SCISEARCH
THE GENUINE ARTICLE: NK091
TITLE: PRODUCTION OF THE IMMUNOGLOBULIN VARIABLE DOMAIN REI(V) VIA **A FUSION PROTEIN** SYNTHESIZED AND SECRETED BY **STAPHYLOCOCCUS** -CARNOSUS
AUTHOR: PSCHORR J; BIESELER B; FRITZ H J (Reprint)
CORPORATE SOURCE: UNIV GOTTINGEN, INST MOLEK GENET, GRISEBACHSTR 8, D-37077 GOTTINGEN, GERMANY (Reprint); UNIV GOTTINGEN, INST MOLEK GENET, D-37077 GOTTINGEN, GERMANY; MAX PLANCK INST BIOCHEM, ZELLBIOL ABT, D-82152 MARTINSRIED, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: BIOLOGICAL CHEMISTRY HOPPE-SEYLER, (APR 1994) Vol. 375, No. 4, pp. 271-280.
ISSN: 0177-3593.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 51

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB REI(v) - the variable domain of an immunoglobulin x light chain - was produced by heterologous gene expression in a Gram-positive bacterium, purified to homogeneity and characterized. A host/vector combination based on secretion of Staphylococcus hyicus lipase by Staphylococcus carnosus was exploited. A gene encoding a fusion protein, composed of an aminoterminal portion of the pre-pro-peptide of S. hyicus lipase, a hexahistidine affinity tag, followed by the recognition sequence of IgA **protease** and REI(v) was constructed. Expression of the fusion gene in S. carnosus causes selective secretion and accumulation of a soluble fusion protein in the culture medium (5-10mg/l), which can be purified from the supernatant by immobilized metal ion **affinity chromatography** (IMAC). REI(v) is released from the fusion protein with an additional threonine and. proline residue at the aminoterminus (REI(v)TP) by site-specific **cleavage** with IgA **protease** and can be separated from the hexahistidine-tagged fusion partner and the **protease** by a second passage through an IMAC gel matrix. Like authentic REI(v), the isolated protein (> 1 mg/l culture medium) migrates as a dimer in gel filtration chromatography and undergoes cooperative, reversible unfolding in urea. The isolated immunoglobulin REI(v)TP and authentic REI(v) have indistinguishable free energies of unfolding (approx. 26 kj/mol, 6.3 kcal/ mel).

L39 ANSWER 17 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 10
ACCESSION NUMBER: 1992:463892 HCAPLUS
DOCUMENT NUMBER: 117:63892
TITLE: A single step purification for recombinant proteins: characterization of a microtubule associated protein (MAP2) fragment which associates with the type II cAMP-dependent protein kinase
AUTHOR(S): Stofko-Hahn, Renata; Carr, Daniel W.; Scott, John D.
CORPORATE SOURCE: Vollum Inst., Oregon Health Sci. Univ., Portland, OR, 97201-3098, USA
SOURCE: FEBS Letters (1992), 302(3), 274-8
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 167 base pair **DNA** cassette has been constructed to facilitate the detection and purification of recombinant proteins. This cassette, kfc, encodes three distinct peptide units: a phosphorylation site for the cAMP-dependent protein kinase (PKA), called kemptide; a factor Xa **cleavage** site; and a calmodulin-binding peptide. Kfc fusion proteins can be purified from bacterial lysates in one step by affinity chromatog. on calmodulin-agarose using EGTA as eluant. As a test of this system, the production, purification, and characterization of the PKA binding domain of the microtubule associated protein (MAP 2) was demonstrated.

L39 ANSWER 18 OF 24 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 93152141 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1369142
TITLE: Continuous production of restriction endonucleases: continuous two-stage cultivation with E. coli JM103; continuous cell disintegration and **purification** by **affinity chromatography**.
AUTHOR: Beer H D; Maschke H E; Schugerl K
CORPORATE SOURCE: Gesellschaft fur Biotechnologische Forschung mbH,

Braunschweig, Federal Republic of Germany.
SOURCE: Applied microbiology and biotechnology, (1992 Nov) 38 (2) 220-5.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19950809
Last Updated on STN: 19950809
Entered Medline: 19930305

AB The optimization of the production of recombinant **DNA**-derived proteins in Escherichia coli was investigated. We chose restriction endonucleases EcoRI and EcoRV from E. coli as model proteins, despite the observation that overproduction can result in a toxic effect to the cells. The enzymes were **expressed** as **fusion proteins** consisting of **protein A** from **Staphylococcus** aureus and the desired enzyme in order to facilitate purification. The expression of the fusion protein was induced by a temperature shift using the pR promoter of phage lambda regulated by the repressor plasmid pRK248cI. Data from batch fermentations provided the basis for planning a continuous two-stage fermentation. The EcoRI enzyme activity was investigated as a function of the induction time after cell disintegration and allowed an estimation of yield of the continuous culture. Plasmid instability, which was only observed under continuous conditions, could be prevented by adding tetracycline (resistance of the repressor plasmid) to the medium. We established a continuous cell disintegration system and **purified** the fusion protein semicontinuously by **affinity chromatography**. The biological activity of the fusion protein was the same as the native endonuclease so there was no need for **cleavage** of the fusion protein and the product could be used without further processing.

L39 ANSWER 19 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1992:484380 HCAPLUS
DOCUMENT NUMBER: 117:84380
TITLE: A method for the evaluation of the efficiency of signal sequences for secretion and correct N-terminal processing of human parathyroid hormone produced in Escherichia coli
AUTHOR(S): Kareem, B. N.; Rokkones, E.; Hoegset, A.; Holmgren, E.; Gautvik, K. M.
CORPORATE SOURCE: Biotechnol. Cent., Oslo, Norway
SOURCE: Analytical Biochemistry (1992), 204(1), 26-33
CODEN: ANBCA2; ISSN: 0003-2697
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Expression plasmids have been constructed for evaluation of different signal sequences for secretion and correct amino terminal processing of foreign proteins expressed in Escherichia coli. A cDNA representing the N-terminal region (1-37) of human parathyroid hormone was inserted between **DNA** coding for 2 different forms of the signal sequence and 2 IgG binding domains (ZZ) derived from Staphylococcal protein A. The expression products were secreted to the periplasm and addnl. into the growth medium and were easily **purified** by **affinity** chromatog. using the ZZ part as a specific handle. Further analyses showed that the expression products were correctly processed to the mature protein hPTH(1-37)ZZ in a construct where the wild type signal sequence of Staphylococcus protein A was used. When a mutated signal sequence which

lacks the normal **cleavage** site was employed, the fusion protein was not **cleaved**. Since signal sequences seem to be processed in the correct way in this system, the general design of this type of expression vector is well suited for studying the N-terminal processing and secretion of heterologous proteins in E. coli.

L39 ANSWER 20 OF 24 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 91:110675 SCISEARCH
THE GENUINE ARTICLE: EY682
TITLE: INTERACTION OF ANDROGEN RESPONSE ELEMENTS WITH THE **DNA**-BINDING DOMAIN OF THE RAT ANDROGEN RECEPTOR EXPRESSED IN ESCHERICHIA-COLI
AUTHOR: DEVOS P; CLAESSENS F; WINDERICKX J; VANDIJCK P; CELIS L; PEETERS B; ROMBAUTS W; HEYNS W; VERHOEVEN G (Reprint)
CORPORATE SOURCE: CATHOLIC UNIV LEUVEN, FAC MED, DEPT BIOCHEM, B-3000 LOUVAIN, BELGIUM; CATHOLIC UNIV LEUVEN, EXPTL MED & ENDOCRINOL LAB, B-3000 LOUVAIN, BELGIUM
COUNTRY OF AUTHOR: BELGIUM
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1991) Vol. 266, No. 6, pp. 3439-3443.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 47

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A fragment of the rat androgen receptor (amino acids 533-637) containing the **DNA**-binding domain was produced in Escherichia coli as **a fusion** product with **protein A** of **Staphylococcus** aureus. The **fusion protein** was purified on IgG-Sepharose, a method that does not involve the use of denaturing agents. Approximately 4 mg of fusion protein was obtained from 500 ml of bacterial culture.

In gel shift assays, the recombinant **DNA-binding** domain displays an **affinity** for a fragment of the long terminal repeat of mouse mammary tumor virus and for an intronic fragment of the gene coding for the C3 component of the androgen-regulated rat prostatic binding protein. In a DNase I footprinting assay, the fusion protein protects a sequence in the C3 fragment that has previously been shown to act as a functional androgen response element. Interestingly, a single base pair mutation in the response element, which abolishes androgen inducibility, also destroys the ability to interact with the recombinant androgen receptor **DNA**-binding domain.

L39 ANSWER 21 OF 24 MEDLINE on STN DUPLICATE 12

ACCESSION NUMBER: 91144529 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1996952
TITLE: Fibroblast adhesion to recombinant tropoelastin expressed as a protein A-fusion protein.
AUTHOR: Grosso L E; Parks W C; Wu L J; Mecham R P
CORPORATE SOURCE: Department of Pathology, Jewish Hospital, Washington University Medical Center, St. Louis, MO.
CONTRACT NUMBER: HL26499 (NHLBI)
HL41040 (NHLBI)
HL41926 (NHLBI)
+
SOURCE: Biochemical journal, (1991 Feb 1) 273 (Pt 3) 517-22.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199103
ENTRY DATE: Entered STN: 19910412
Last Updated on STN: 19910412
Entered Medline: 19910327

AB A bovine tropoelastin cDNA encoding exons 15-36 that includes the elastin-receptor binding site was **expressed** in Escherichia coli as **a fusion protein** with **Protein A** from **Staphylococcus** aureus. After isolation of the fusion protein by **affinity chromatography** on Ig-Sepharose, the tropoelastin domain was separated from plasmid-pR1T2T-encoded Protein A (Protein A') by CNBr **cleavage**. Cell-adhesion assays demonstrated specific adhesion to the recombinant tropoelastin. Furthermore, the data indicate that interactions involving the bovine elastin receptor mediate nuchalligament fibroblast adhesion to the recombinant protein. In agreement with earlier studies of fibroblast chemotaxis to bovine tropoelastin, nuchal-ligament fibroblast adhesion demonstrated developmental regulation of the elastin receptor.

L39 ANSWER 22 OF 24 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 89093147 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2642905
TITLE: High level expression in Escherichia coli of the **DNA**-binding domain of the glucocorticoid receptor in a functional form utilizing domain-specific **cleavage** of a fusion protein.
AUTHOR: Dahlman K; Stromstedt P E; Rae C; Jornvall H; Flock J I; Carlstedt-Duke J; Gustafsson J A
CORPORATE SOURCE: Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital, Sweden.
SOURCE: Journal of biological chemistry, (1989 Jan 15) 264 (2) 804-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198902
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19890217

AB A fragment comprising the **DNA**-binding domain of the human glucocorticoid receptor has been expressed in a functional form in Escherichia coli as **a fusion protein** with **protein A** from **Staphylococcus** aureus. The **DNA**-binding domain was **purified** to apparent homogeneity by **affinity chromatography** on IgG-Sepharose and **DNA**-cellulose, a purification scheme which does not involve denaturation of the protein at any step. The **DNA**-binding domain was separated from the protein A part of the fusion protein by domain-specific enzymatic **cleavage** with chymotrypsin while immobilized on IgG-Sepharose. The recombinant protein has been characterized by amino acid analysis, NH2- and COOH-terminal sequence analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and reactivity to iodoacetate and was found to correspond to the primary structure derived from the cDNA sequence. DNase I footprinting showed that the purified recombinant protein bound to the same **DNA** sequences on the mouse mammary tumor virus long terminal repeat as glucocorticoid receptor purified from rat liver does. About 10 times more

recombinant protein, on a molar basis, was needed to obtain the same level of protection. However, the protection of the three different footprints (1.3, 1.4, and 1.5') by the recombinant protein differed greatly from that of the natural receptor, with virtually no protection of footprint 1.4. This indicates cooperative binding of the natural receptor to adjacent footprints, dependent on other regions of the receptor than the **DNA**-binding domain.

L39 ANSWER 23 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1988:491125 HCAPLUS
DOCUMENT NUMBER: 109:91125
TITLE: Genetic approaches to protein purification
AUTHOR(S): Uhlen, Mathias; Abrahmsen, Lars; Moks, Tomas; Nilsson, Bjoern
CORPORATE SOURCE: Dep. Biochem., R. Inst. Technol., Stockholm, 100 44, Swed.
SOURCE: Makromolekulare Chemie, Macromolecular Symposia (1988), 17(Int. Symp. Affinity Chromatogr. Interfacial Macromol. Interact., 1987), 483-9
CODEN: MCMSES; ISSN: 0258-0322
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 18 refs. A gene **fusion** system based on the **protein A** gene from **Staphylococcus** aureus has been developed to facilitate purification of recombinant proteins, both in large and small scale. Due to the strong interaction with IgG, it is possible to recover gene products fused to various protein A derivs. in a one-step procedure with high yield and in purity. Site-directed mutagenesis is used to introduce enzymic and chemical **cleavage** sites at the fusion point between the protein A derivative and the desired protein. The protein A "tail" can thereby be removed from the **affinity purified** fusion protein by the appropriate **cleavage**, releasing biol. active mols. Recently, the system was improved by designing a synthetic **DNA** fragment encoding two IgG-binding domains derived from staphylococcal protein A which are resistant to various chemical **cleavages**. The gene fusion product is secreted to the culture medium of Escherichia coli and can be recovered simply by passing the clarified culture medium through an IgG Fast Flow Sepharose. The system has been used to immobilize enzymes, to obtain monoclonal and polyclonal antibodies, and to produce biol. active human peptide hormones in pilot plant scale.

L39 ANSWER 24 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1985:107464 HCAPLUS
DOCUMENT NUMBER: 102:107464
TITLE: Producing and selectivity isolating proteins and polypeptides, recombinant and expression vector therefor and fusion protein able to bind to the constant region of immunoglobulins
INVENTOR(S): Loefdahl, Sven; Uhlen, Mathias; Lindberg, Martin; Sjoequist, John
PATENT ASSIGNEE(S): Pharmacia AB, Swed.
SOURCE: PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 8403103	A1	19840816	WO 1984-SE46	19840209
W: JP, US				
RW: AT, BE, CH, DE, FR, GB, LU, NL, SE				
SE 8300693	A	19840810	SE 1983-693	19830209
EP 135532	A1	19850403	EP 1984-900773	19840209
EP 135532	B1	19880810		
R: AT, BE, CH, DE, FR, GB, LI, LU, NL, SE				
JP 60500480	T2	19850411	JP 1984-500885	19840209
JP 06034742	B4	19940511		
AT 36348	E	19880815	AT 1984-900773	19840209
US 5100788	A	19920331	US 1988-196846	19880509
JP 07265078	A2	19951017	JP 1995-117618	19950420

PRIORITY APPLN. INFO.:	SE 1983-693	A	19830209
	EP 1984-900773	A	19840209
	WO 1984-SE46	W	19840209
	US 1984-667492	B1	19841009

AB A **DNA** sequence coding for **protein A** (an IgG-binding **protein** from **Staphylococcus** aureus) is **fused** with a **DNA** sequence coding for a desired protein and is cloned in a plasmid or phage vector for expression in a bacterium. The fusion protein formed is recovered by affinity chromatog. using immobilized IgG on a support. The support-bound fusion protein may be used as is, or the desired protein may be cloned at its junction with protein A and released from the support. Thus, **DNA** from plasmid pSPA5 containing the protein A gene and plasmid pBR322 **DNA** was ligated to **DNA** of plasmid pUR22 containing a gene for β-galactosidase [9031-11-2] by conventional methods, and the ligated mixture was used to transform Escherichia coli. Transformants were recovered from X gal (chromogenic 5-bromo-4-chloro-3-indolyl-β-D-galactoside) plates containing ampicillin and tetracycline. Plasmid pSPA10 with the protein A gene fused to the lacZ' gene at its Sau3A site at position 1096 was recovered. The protein A-encoding fragment has a unique EcoRI site adjacent to its downstream end, at which a **DNA** linker containing multiple restriction sites was attached. Expression of the recombinant plasmid in E. coli resulted in formation of a fusion protein. The fusion protein was passed over an IgG-Sepharose 4B column and demonstrated to be β-galactosidase by color reaction, but elution with glycine buffer inactivated the enzyme. Similarly, a fusion gene encoding protein A and human insulin-like growth factor 1 (IGF-1) [67763-96-6] was inserted on a plasmid, used to transform E. coli, and IGF-1 was **purified** by IgG **affinity** chromatog. and treatment with formic acid [64-18-6], which **cleaves** the protein at the aspartic acid-proline dipeptides. The IGF-1 protein recovered locked the N-terminal glycine and had an activity of 143 units/L.